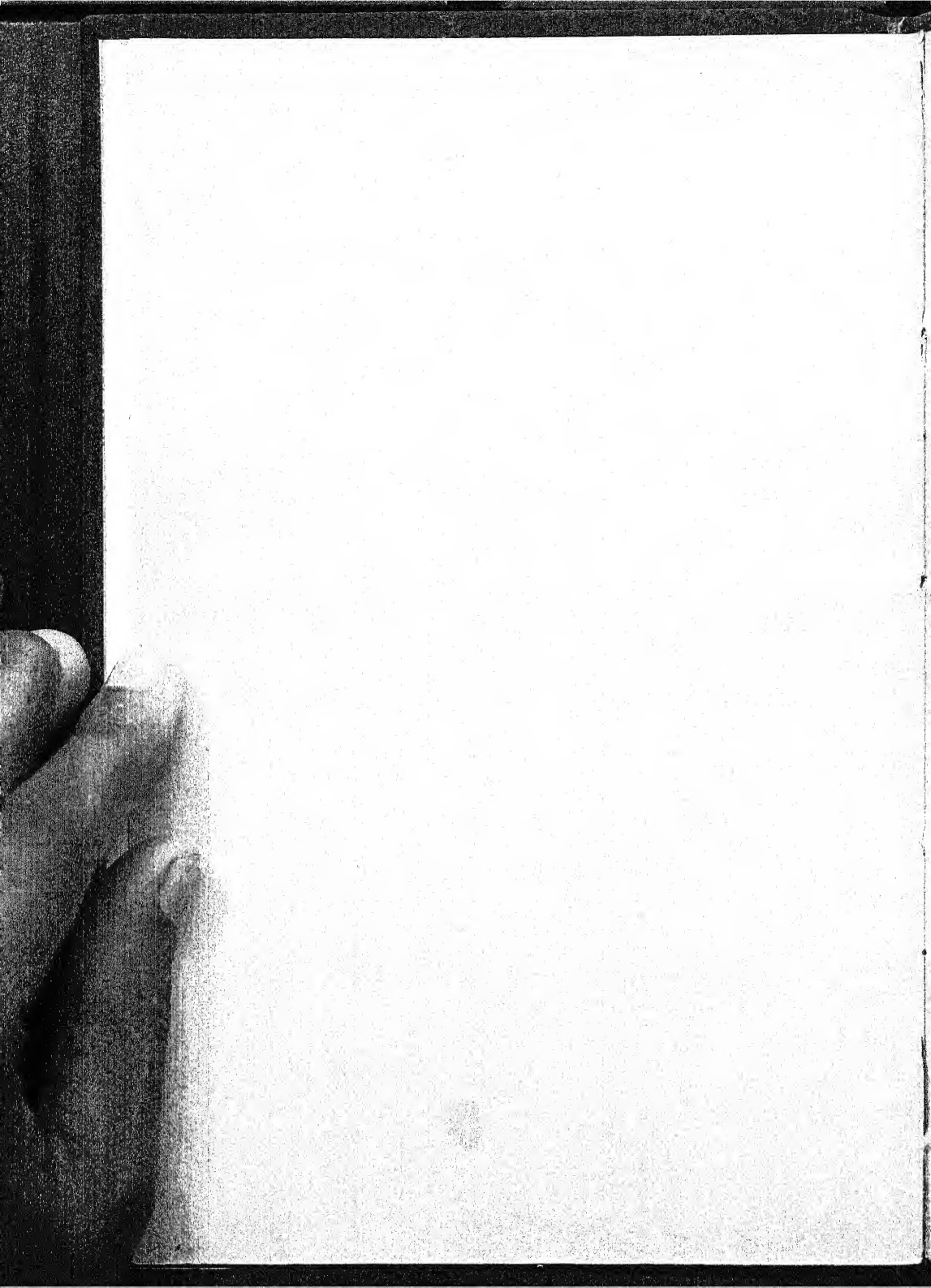
Photomicrography

An Introduction to Photography with the Microscope, with Notes on the Visual Use of Light Filters in Microscopy

Seventh Edition

Kodak Limited, Kingsway, London, W.C.2.



INTRODUCTION

The wide use of photographic materials in connection with microscopy for pathological, medical, metallurgical and many other purposes makes desirable a booklet dealing with the photographic problems involved in photomicrography.

For some years our Wratten division has specialised on the preparation of sensitive material and colour-filters for use in photomicrography and has issued a series of booklets dealing with this subject. The present booklet, however, represents a complete revision of those previously published, and we offer it in the hope that it will be of use to those of our customers who are interested in photography through the microscope.

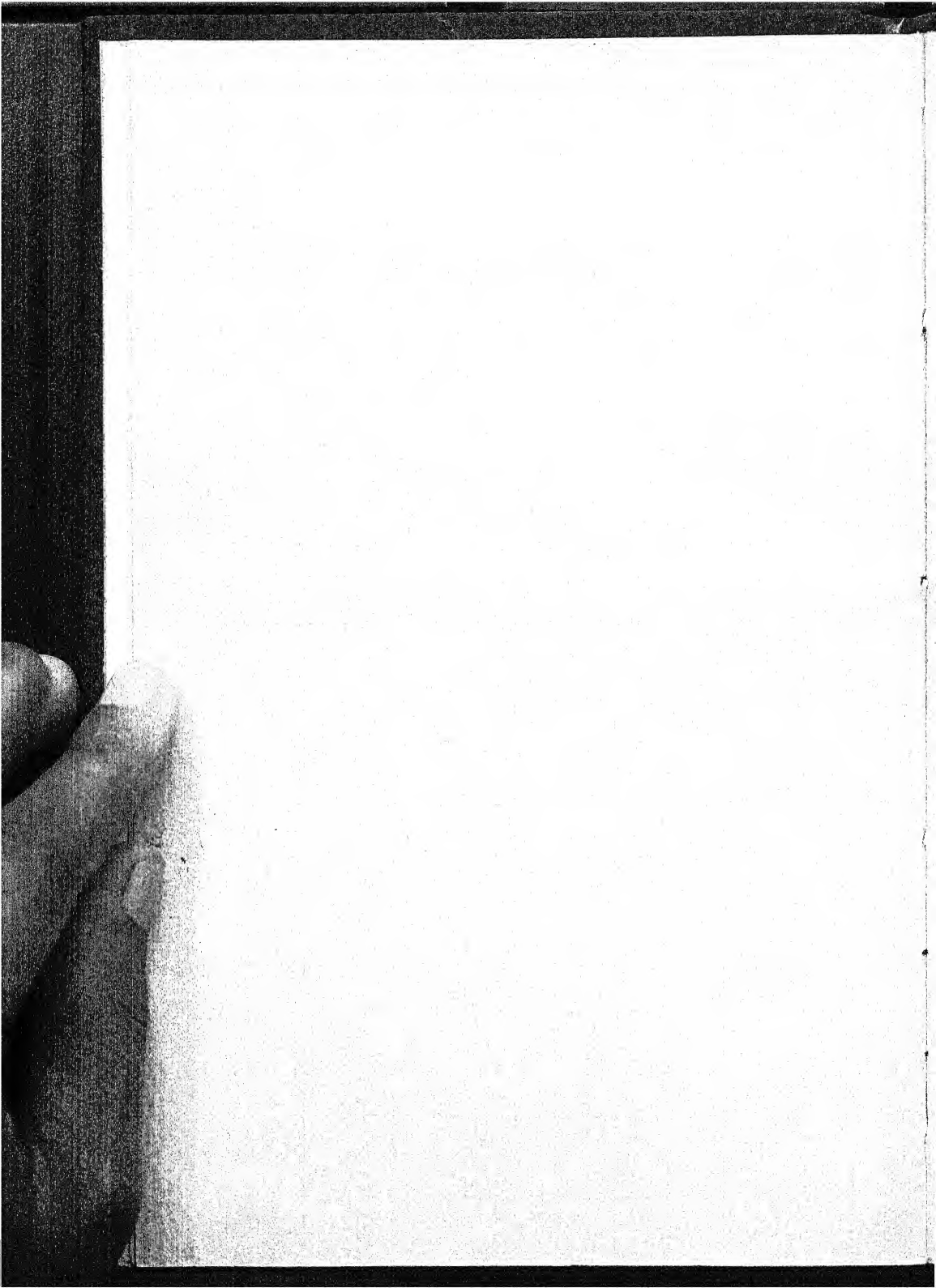
The style is intended to be expository, not scientific. We are less concerned to discuss possible methods than to explain the easiest, and while some of the statements made need qualification, we have preferred to offer them without qualification rather than perplex the reader.

This booklet is not intended to replace the regular text-books of the subject, but to supplement them. For example, we might mention as giving a much fuller treatment of the subject, Messrs. Hind & Randles' "Handbook of Photomicrography," Bagshaw's "Elementary Photomicrography" (third edition), Barnard's "Practical Photomicrography," and Dr. Spitta's "Photomicrography." It is also especially worth while for a photomicrographer to master the theory of the microscope, for which purpose we can recommend Dr. Spitta's "Microscopy" (third edition, 1920).

KODAK, LTD.

KINGSWAY,
LONDON, W.C.2.

March, 1926.



Photomicrography

APPARATUS

The apparatus arranged for photomicrography should fulfil two requirements: It should be rigid and rigidly connected together so that any vibration will affect the whole and will not shake one part relatively to the rest; and the source of light, condensing system, microscope and camera, should be capable of being fixed accurately in a straight line, so that they will stay in position. Provided that these requirements are fulfilled, elaborate apparatus is not a necessity and the simplest apparatus may do good work. It is recommended, however, that a photographer who has much photomicrography to do should obtain apparatus built on a properly made optical bench, solidly constructed and in which he can vary easily the extension of the camera and the distance of the light source from the microscope. In addition to the large benches built by the optical instrument makers there are a number of simpler instruments designed for special purposes. Thus, some makers produce convenient and light apparatus taking a very small film or plate (4.5 × 6 c.m.), and intended to be fitted on the eyepiece end of a vertical microscope. Other specialized apparatus is designed solely for the photomicrography by reflected light of polished and etched metal surfaces or similar preparations.

Of the microscopes themselves, little need be said except that only a first-class microscope is really suitable for photomicrography. It should be very rigid and heavy, and it is almost essential that it should be fitted with a mechanical stage. If the microscope, which it is proposed to use, be deficient in this respect it is desirable to fit one of the detachable mechanical stages which are on the market.

LIGHT SOURCES

For photomicrography an electric source of light is undoubtedly best. Possibly the Ediswan Pointolite should have pride of place. It is available in two forms—one for use on direct and one for alternating circuits. That for direct current is to be preferred. When the alternating current model is employed the tungsten spheres should be in the optic axis and one behind the other, so that the image of one only is formed by the collecting lens. Arcs of various types are much used and have many advantages and some disadvantages. The quartz mercury vapour lamp is of great value in special work, and several models recently placed on the market make it also generally useful. For low powers, small, metal-filament lamps, preferably with concentrated filaments and of the half watt, gas-filled type, run from accumulators or through transformers are useful, especially for the direct lighting of opaque preparations. For those who

have no electric supply the thorium pastille incandescent gas lamp is very convenient ; it is of high actinic (and visual) power, and is extremely economical in use. Last and by no means to be ignored is the oil lamp, always ready and self-contained. With a one-inch wick and a good collecting system—oil immersion objective and oil condenser, the exposure required with Eastman Commercial Panchromatic film at 1,000 diameters, no light filter, is from 10 to 15 seconds with an average preparation or diatom.

For very low power work—up to 3 or 4 diameters—daylight is sometimes employed, if the apparatus is conveniently placed, and preferably of the vertical type. Magnesium ribbon has its uses for similar work. Direct sunlight, fed to the microscope by means of a heliostat, can only be employed in countries more favoured with steady sunshine than is the case here.

GENERAL OPTICAL PRINCIPLES

The compound microscope, as it is termed in text books dealing with optics, consists essentially of two portions, the objective and the eyepiece. In the diagram :

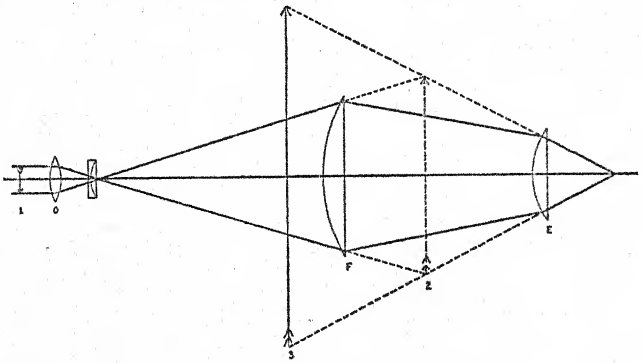


FIG. 1

the objective at O would form an image at 2 of the small object marked 1, this image, of course, being inverted and being a real image, but before the real image can be formed the rays are intercepted by the eyepiece lens F, which in combination with the second eyepiece lens E, forms a magnified virtual image 3, corresponding to the real image 2, so that the original object undergoes two stages of magnification, one by the objective and the other by the eyepiece. The magnifying power of the microscope is therefore the magnifying power of the objective multiplied by that of the eyepiece.

The following table gives approximately the magnifying power of an objective used alone (Col. 3), and with eyepieces of known magnifying power at 200 mm. tube length (Cols. 4, 5 and 6) :

Equivalent Focal Length.		Initial Magnifying Power with Image Distance 10 in.	Eyepieces with Initial Magnifying Power		
in.	mm.		× 2	× 5	× 10
2	50	5	8	20	40
1	25	10	16	40	80
½	12	20	32	80	160
¼	6	40	64	160	320
⅛	3	80	128	320	640
1/12	2	120	192	480	960

The magnification for objective plus eyepiece is calculated for an image distance of 10 inches (250 mm.) from eyepiece to focussing screen. It is recommended that calculated magnifications should be checked by actual measurements of the projected image of a stage micrometer.

(*Note.*—Most one-twelfths are really one-fourteenths—with consequent increase of magnification).

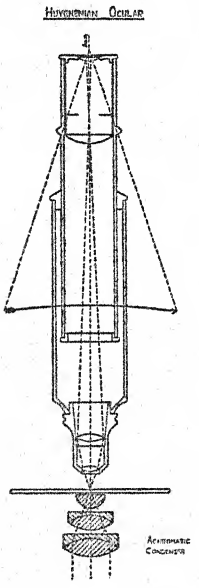


FIG. 2

Figure 2 shows the whole system of the microscope fitted with a Huyghenian eyepiece and with an achromatic condenser. It will be seen that the light entering the condenser converges at the object, diverges towards the objective and then passing through the objective converges again at the diaphragm of the eyepiece so that the eye looking into the eyepiece sees a virtual, enlarged image of the object appearing to be about half-way down the tube of the microscope.

Now, it is clear that we could get any degree of magnification we require by simply using a long enough tube or high enough powers of eyepiece, and there would seem at first sight to be no limit to the fineness of detail which we could perceive with the microscope since we could easily use another microscope instead of an eyepiece and so get almost unlimited magnification. Unfortunately, while we could easily obtain any magnification in this way, the amount of detail which can be observed is quite strictly limited by certain optical laws, and mere magnification of the image does not enable us to see

more detail than is given by the construction of the objective. An eyepiece of ten or a magnification of about ten times the magnification given by the objective will usually enable us to see all the detail which the objective can give. This limit to detail or, as it is generally called, to the resolving power of a microscope, is fixed by the structure of light itself since light is not a continuous flow of substance but consists of definite waves having a definite length which make, so to speak, a structure to the light so that we cannot hope to see things which are much smaller than the structure of the light itself.

The resolving power, or the distance by which two small elements in an object must be separated in order to be visible depends upon the numerical aperture (N.A.) of the objective, the higher the N.A. the greater the resolving power of the objective and the finer the detail it can reveal. Numerical aperture is equal to the effective aperture of the back lens of the objective divided by twice the equivalent focus. Now, if a very narrow central pencil is used for illumination the finest detail that can be shown by a microscope with high enough magnification is equal to $\frac{w.l.,}{\text{N.A.}}$ where $w.\ l.$ is the wave length of the light used for illumination. The wider the pencil used for illumination the greater the resolving power until a maximum is reached, when the whole aperture of the objective is full of light, and in this case the resolving power is twice as great as with a narrow pencil and is equal to $\frac{w.l.}{2\,\text{N.A.}}$. This same limit is reached when a narrow pencil entering the lens as obliquely as possible is used. The wave length of light can be taken as being half 1/1,000th of a millimetre, which is about 1/50,000th of an inch, so that a lens in which the effective aperture of the back lens is equal to the equivalent focus and in which consequently the numerical aperture is equal to $\frac{1}{2}$, can separate lines which are 1/25,000th of an inch apart if all the back lens is filled with light, but with a narrow pencil the lines must be 1/12,500th of an inch apart in order to be resolved with this objective.

The numerical aperture of the lenses used in photomicrography is generally very considerable, the highest power objectives having numerical apertures above 1·0, so that they will resolve 50,000 lines to the inch.

When we try to use such large apertures, we are met with a difficulty. In microscopy we are mostly looking through a sheet of glass, this being the cover glass over the specimen, and a beam of light which is coming through glass cannot get out of the glass at all if it meets the surface at an angle greater than a certain fixed amount. This holds true of all micro objectives with a numerical aperture greater than 1; and in

order to use such lenses it is necessary to place between the objective and the cover glass a little pool of oil of the same refractive index as the glass so that no resistance is offered to the path of the ray. Objectives which are used in this way with oil between the front lens and cover glass are called oil-immersion objectives, and by means of oil-immersion it is possible to get apertures as high as 1·4, such an aperture enabling us, under the most favourable conditions, to separate two lines which are as close together as 1/100,000 of an inch, or even a little closer. Oil-immersion substage condensers are a still greater refinement and are similarly used.

In order to make full use of these high aperture objectives, however, it is necessary to arrange the illumination so that the full aperture of the objective is utilized, as if the beam entering the objective from the condenser is not of sufficiently wide angle, the aperture of the objective is of no use and only a portion of the possible resolving power will be obtained. The conditions for obtaining full resolving power are (1) that the whole aperture of the objective be filled with light, and (2) that "critical" illumination be used.

This term "critical" illumination is much discussed in microscopy, but in reality it is quite simple both in its fundamental theory and in practice. The condition for obtaining critical illumination in theory is that all the waves of light reaching the object and forming the image at any one instant of time leave the source of light also at the same instant of time and from the same point. This condition is fulfilled absolutely, of course, if the source of light is focussed on the object, but it is also fulfilled with a fair approximation to exactness if we focus on the object any section of the beam which is reasonably free from aberration.

One arrangement is to use one or two lenses between the source of light and the substage condenser.

One lens can be used to form an image of the arc crater at the back of the condenser, thus filling the condenser with light, the lens near the arc generally being fitted with an iris diaphragm. If such a large image of the crater is not required, two lenses may be used, the size of the image of the crater produced depending upon the focal lengths of these lenses, while when using an incandescent gas mantle or similar large source of light, the best arrangement is such that the lenses are placed so as to project upon the back of the condenser a somewhat diminished image of the large source of light.

For a full description of another very good method of illumination in photomicrography, see an article by Dr. D. J. Reid

in " British Journal Photographic Almanac, 1915," and a further article by the same writer in " Journal Photomicrographic Society, 1921, February."

If the image of the light source just fills the opening in the condenser, then with a given aperture of objective and condenser the brightness of the image on the focussing screen depends simply on the intrinsic brightness of the source of light and not at all on its size so that, for instance, a properly arranged 4 ampere arc gives the same brightness as a 30 ampere arc, because the increased current simply increases the area of the crater of the carbon and not its brightness. With lower powers, however, the larger crater of the 30 ampere arc is an advantage because it is very difficult to get a large enough evenly illuminated field with the small arc if it is focussed on the condenser iris diaphragm.

ADJUSTMENT OF THE APPARATUS

Let us take up the question of adjusting the microscope for practical work. The apparatus is arranged and fastened rigidly to its support. The object to be photographed is placed on the stage and roughly focussed, whether visually with an eyepiece in the tube, or, if more convenient, with or without eyepiece, on the ground glass of the camera. If we are not using an eyepiece it may be found that the focussing screen has all sorts of curious reflections on it. If we open the camera back and look down the microscope tube we shall see the cause. The inside of a microscope tube is often bright. In this case we must make a velvet tube with the pile inside and line the microscope tube with it. This will remove the reflections completely. The ground glass of the camera should have a $\frac{3}{4}$-inch circular cover glass cemented to the centre of the ground glass side with Canada Balsam, a pencil cross having been made on the ground glass before cementing. This enables the final fine focussing with a magnifier to be done with exactitude.

Centreing

Roughly focus an object placed on the microscope stage, using, preferably, a low power eyepiece. Close down to its limit the iris of the substage condenser and by means of its rack or other adjustment get the image of the small remaining aperture of the iris sharply defined in the field. Centre this image and open up the iris. Rack up the substage condenser until an image of the light source is focussed in the field and centre this image by moving the light source. A collecting-lens (parallelizer) or auxiliary condenser being almost invariably used in photomicrography, this should now be placed in position and its iris

closed down. (If no iris be fitted a detachable metal cap should be used having exactly in its centre a small hole say 2 mm. diameter.) The image of the iris (or small hole) is centred in the microscope field by moving the collecting lens, and the iris opened until the image of its aperture formed by the substage condenser just clears (is slightly larger than) the eyepiece diaphragm, or, if no eyepiece is being used, until the iris image on the focussing screen just clears the desired field.

Aperture of the Substage Condenser Iris

It is important that the diaphragm of the substage condenser should not be open to a larger extent than is necessary, as the light scattered by the object from the external and uselessly lighted portions of the object degrades the definition in the field. Theoretically, the definition should be good if the image be carefully focussed, but, as a matter of fact, the definition, especially if the objective is not of the very best, may be poor, the image appearing obscured by scattered light. The iris diaphragm of the microscope condenser is, therefore, closed until this scattered light disappears and the image appears sharp and clear. The aperture of the diaphragm should not be smaller than is absolutely necessary, because beyond a certain point, closing the diaphragm spoils the definition instead of improving it. With perfect objectives the definition is best with the largest openings.

Low Power Work

For low power work in photomicrography, say up to 10 diameters, with the short-focus anastigmats especially designed for the work it will often be found more convenient to dispense with the microscope stand and attach the lens directly to the front of a long-extension camera, the preparation being held in a special stand. Focussing may be done by having the lens in a focussing mount, by moving the camera, or, perhaps best, by moving the stand which holds the object. For illuminating large objects by transmitted light a plano-convex lens of, say, about 5 inches focal length and somewhat larger in diameter than the longest dimension of the object should be placed behind and close to the preparation, plane side to the object. The optical arrangement is practically the same as when enlarging a photographic negative. It is usually not possible to bring the light source near enough as one would in an enlarging lantern, but with the plano-convex lens in position, adjust the position of the collecting lens relatively to the light source so that a sharp image of the collecting lens iris, or light source, formed by the plano-convex lens, is thrown on to a slip of white card placed

just in front of the micro. objective or photographic lens in use. The image of the collecting lens iris should just fill the front lens of the objective. An iris fitted just behind the preparation and adjusted so that only the field to be photographed is illuminated, is very useful in helping to eliminate haze.

DARK-GROUND ILLUMINATION

One of the most useful methods of illuminating certain objects is what is known as dark ground illumination. This can be secured by supporting the objects themselves on a dark ground and illuminating them from above, but small objects are best dealt with by another method. The principle of the method is made clear in Fig. 3.

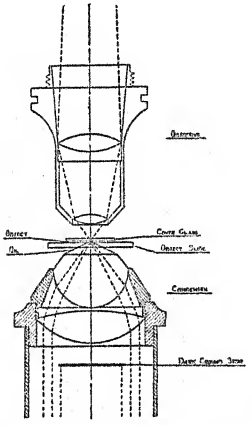


FIG. 3

A very high-aperture condenser is used which is optically connected to the slide by a drop of oil. The objective used is of an aperture smaller than that of the condenser and then a " dark field stop " is placed below the condenser, so that it stops out a central area equal to the aperture of the objective and no light whatever goes straight through from the condenser iris into the objective; thus, a dark field is obtained. The darkness of the field depends chiefly on the freedom of all the lenses from dust or from bright places capable of scattering light.

Now, if an object be on the stage, it will be brightly illuminated by the oblique rays from the edges of the condenser and will bend some of these so that they enter the objective and the object will appear bright on the dark ground.

The intensity will depend on the amount of light reaching the object, that is, on the aperture of the outer cone of light from the condenser. If the aperture of the whole condenser be C_A and that of the objective be O_A, then the dark field stop must stop out O_A, from C_A and the brightness of the cone will be $C_A—O_A$.

As O_A is much higher in high power objectives than in low ones, and as also the illumination will decrease as the square of the magnification, this method has been restricted to low-power work until recently, because it is very difficult to get

even illumination over a large field with a small light source if it be focussed on the condenser iris.

The newer dark-ground illuminators, however, give more light and can be satisfactorily used with high power objectives. (See Fig. 4.) But with these it is usually necessary to employ a funnel stop in the objective to reduce its N. A. to about 0·7.

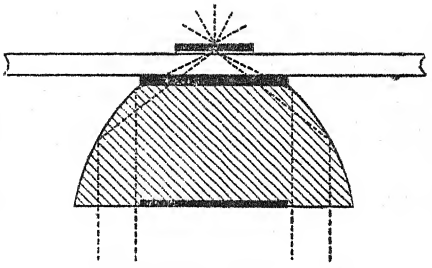


FIG. 4

CONTRAST

As the object of taking photomicrographs is to exhibit as clearly as possible the structure of the specimen, the subject really resolves itself into the rendering of, and intensifying, or diminishing, contrast. The contrast of different specimens varies greatly, but in photomicrography the difficulty chiefly arising is that the contrast between the structure to be shown and its background is insufficient. This is the case, for instance, in lightly stained sections, in diatoms, etc. Strongly stained sections present no difficulties when photographed as a whole against a clear background, but if details of the structure are to be distinguished from the mass by the aid of a high power, there may be insufficient contrast.

The Causes Preventing Sufficient Contrast

Sometimes insufficient contrast may be produced by unsatisfactory lighting, an unnecessarily large aperture in the collecting lens, or a larger condenser stop than the objective will bear will tend to produce it. Dirty lenses will also spoil the whole image, covering it with scattered light ruinous to all contrast. But the main control of contrast will be found by the control of the colour of the light used for illumination, and it is for this control that we have devised our special system of filters used with plates sensitive to all colours.

The Nature of Light and Colour

When ordinary light is analyzed by means of the instrument known as the spectroscope, it is found to consist of a mixture of different kinds of light which, falling upon the eye, produces various sensations which we term sensations of colour. The analysis of daylight or arc light shows a continuous band of colours which appears to consist of three main portions, blue-violet, green and red, the blue-violet passing through gradations

of blue and blue-green to green, and the green through gradations of yellow and orange to red. If an object does not equally reflect or transmit all the different kinds of light of which the white light is composed, the light from it falling upon the eye will be more or less wanting in some constituents and will produce a sensation of colour; so that a coloured object is one which does not equally reflect or transmit all the constituents of white light, but which "absorbs" some. The light which is absorbed is usually converted into heat and helps to warm the coloured object.

If we analyze in a spectroscope the light reflected by a coloured object, or transmitted by a coloured "filter," we shall find that the continuous spectrum which is obtained with white light is replaced by one from which a portion is partly or completely missing. This missing portion appears as a black band, which is generally known as the "absorption band" of the colour. If a particular object absorbs most of the constituents from white light so that only a small portion of the spectrum is transmitted, that portion may be referred to as the "transmission band."

As it is the light which is not absorbed that falls upon the eye, the sensation of colour produced is the reverse of, or "complementary" to, the colour which is absorbed.

If objects of various colours are examined it will be found that a light blue object has an absorption band in the red, a purple object in the yellow, a magenta in the green, an orange in the blue-green, and a yellow in the blue-violet. Thus a sensation of "light blue" is produced by a mixture of green light and blue-violet light falling upon the eye, the red light being more or less absent, having been absorbed from the white light by the object, which appears to be coloured light blue. In the same way a sensation of "yellow" is produced by a mixture of green light and red light falling upon the eye, the blue-violet light having been absorbed.

If the blue-violet light is

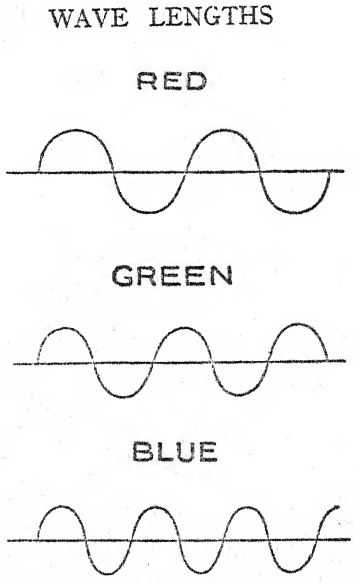


FIG. 5

deficient in the light source, the original light will appear yellowish as does gaslight for instance.

The Definition of Colour

Light is known to consist of waves, and the colour of the light is connected with the length of the waves. The length of a light wave is the distance from the crest of one wave to the crest of the next, measured in very small units (the Angström unit is one ten-millionth of a millimetre).

A wave of the darkest violet that we can see will be 4,000 of these units in length, a wave of blue-green 5,000 units, of bright green 5,500 units, of orange 6,000 units, and of deep red 7,000 units (see Fig. 5). So that visible light is composed of light waves, varying in length from 4,000 to 7,000 units, which may be divided roughly into three portions: blue-violet 4,000-5,000 units; green 5,000-6,000 units; red 6,000-7,000 units (see Fig. 6).

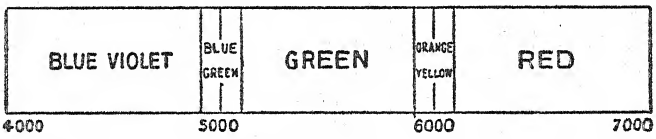


FIG. 6

The position of an absorption band may be defined by the length of the waves of light which are absorbed by it, and we may speak of an absorption band as extending, for instance, from 6,000 to 6,400 units, meaning thereby a band absorbing those particular waves of red light, and therefore producing a blue colour.

Colours Produced by a Single Absorption Band

In order to realize the relation between absorption and the colour of the object it may be worth while to examine the effect of a single sharp absorption band in different parts of the spectrum. First consider a sharp absorption band in the extreme red, stretching from 6,400 to 6,900, and completely stopping all red light of those wave lengths. The remaining colour will consist of all the blue-violet and all the green light, with some of the red. The actual visual effect of a mixture of the light left, what one might call the *residual* colour, is a sky-blue. Imagine this band to shift so that the absorption is between 5,800 and 6,200, the residual colour will be a light blue-violet because there is a great deal of red being transmitted and less green. If the band shifts into the yellowish green, from 5,600 to 6,000, it will absorb a great deal of the green and no red, and the residual colour will become a bluish-purple. As it shifts lower in the green towards the blue this purple becomes a reddish-purple, so that when the band is situated at from 5,200 to 5,600

we have a magenta colour. As the band shifts towards the blue the magenta becomes more orange, and then as the band moves into the blue-violet the orange becomes a yellow and finally a lemon-yellow (see Fig. 7).

Therefore when anything is coloured sky-blue it means that it is absorbing the deep red, a violet-blue object absorbs the orange, a purple the yellow-green, a magenta the central green, an orange the blue, a yellow the blue-violet, and a lemon-yellow only the extreme violet. If a sky-blue object be looked at through a piece of yellow glass it will be found to look bright green in colour, so that a green colour is produced by the absorption both of the red and of the blue, the blue object absorbing the red light and the yellow glass the blue light.

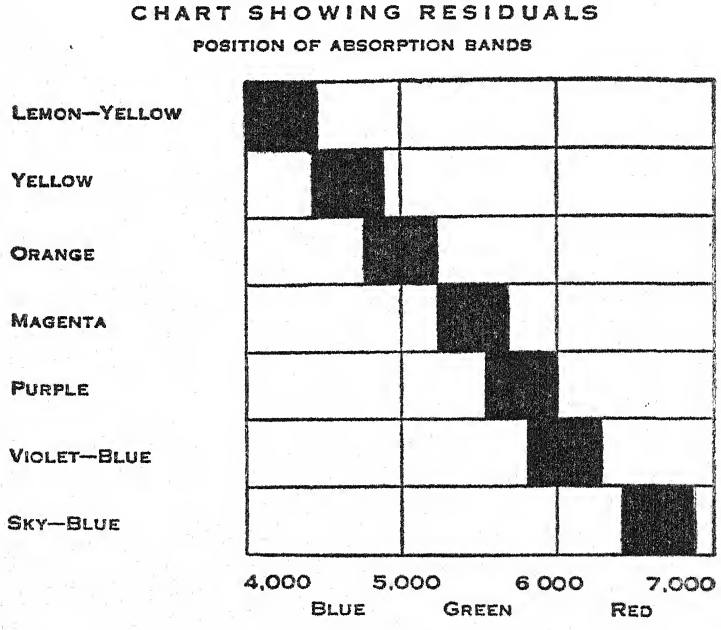


FIG. 7

Natural colours do not generally show sharp absorption bands, though the absorption bands produced by the stains used in microscopy are mostly fairly sharp. The same rule holds true, however; if a magenta object in nature does not signify a clean, sharp absorption band in the green, it still means that that object absorbs far more of the green than of any other colour, and we can apply the rules deduced from

theoretical residuals to natural colours. The first of these rules is as follows:

If a colour is to be rendered as black as possible, then it must be viewed, or photographed, by light which is completely absorbed by the colour, that is, by light of the wave-lengths comprised within its absorption band.

Contrast Within the Object Itself

The second rule for procedure deals with the case where contrast is required; not against the background but within the object itself.

A good case of this is the photography of an unstained section of whalebone; this is of a yellow colour and shows ample detail to the eye, but it completely absorbs blue-violet light, and if it is photographed on an ordinary plate sensitive only to blue-violet light, then it shows far too much contrast appearing as a black detail-less mass against the background, and presents an exaggerated example of the loss of detail occurring when a section is photographed by light which it completely absorbs.

The proper procedure in this case is to photograph the object by the light which it transmits. The whalebone section, for instance, photographed by red light gives perfectly satisfactory results, showing ample detail in structure.

The class of microscopic objects which requires this treatment is that of the usual insect preparations, which, when photographed by yellow or red light, will give quite satisfactory results.

How to Decide on Procedure

The best method of determining the contrast required by any object is to examine the object visually under the microscope first by means of a combination of filters transmitting as completely as possible light of the wave-length absorbed by the preparation, and then by other filters transmitting light less completely absorbed, until the degree of contrast obtained is satisfactory to the eye; as a guide to procedure the next section contains a list of the chief microscopic stains and of the filters which will produce the maximum contrast.

THE WRATTEN "M" FILTERS

In order to enable microscopists, and especially those who are photomicrographers, to control easily the colour of the light they use, the firm of Wratten issued in 1907 a set of nine light-filters, known as the Wratten "M" Filters. The set has been several times revised and improved, and in its present form is generally acknowledged to be indispensable to all serious

workers. The "M" Filters, like all our filters, are prepared originally as gelatine film, stained with appropriate dyes selected for their brightness, sharpness of absorption and stability.

A full description of all our filters, showing their spectral characteristics, will be found in a special book on the subject entitled "Wratten Light Filters."

The "M" Filters can be obtained either as gelatine film, in which case they should be protected from damage by the fingers by binding them up between two pieces of glass, or by cementing with Canada balsam between two sheets of white optical glass, the cementing protects the gelatine filter and gives increased transparency.

The set consists of the following filters:

Name of Filter.	Visual Colour.	Spectral Transmission.
A - 25	Orange Red	From 5,800 to red end
B - 58	Green	,, 4,600 to 6,000
C - 49	Blue-Violet	,, 4,000 to 5,100
D - 35	Purple	,, 3,800 to 4,600 and from 6,400 to red end
E - 22	Orange	,, 5,600 to red end
F - 29	Pure Red	,, 6,100 to red end
G - 15	Strong Yellow	,, 5,100 to red end
H - 45	Blue	,, 4,200 to 5,400
Kl - 6	Pale Yellow	Luminosity filter for orthochromatic reproduction with artificial light

By using these filters in pairs the spectrum can be divided up into approximately monochromatic portions.

Filters	Dominant Wave Length	Colour
D and H	4,500	Violet
C ,, H	4,800	Blue
B ,, C	5,050	Blue-Green
B ,, H	5,200	Bluish-Green
G ,, H	5,350	Pure Green
B ,, G	5,500	Yellowish-Green
B ,, E	5,750	Greenish-Yellow
A	6,250	Orange-Red
F	6,500	Red
A and D	6,750	Deep Red

Visual Inspection the Best Guide

As has already been explained, it is not possible to give instructions for all cases, and the best guide to the combination of filters to be used in any particular case is the visual inspection of the objects through the filters and, of course, the use of the combination with which the degree of contrast or detail, as the

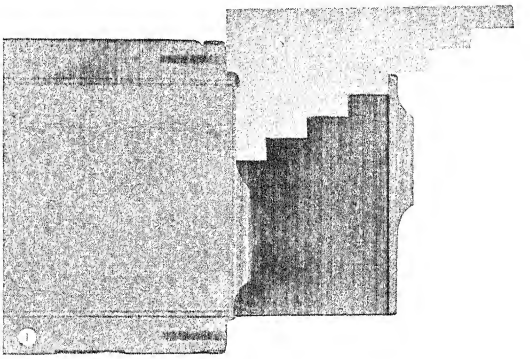


FIG. A.

Cardboard cut for use with test strips. (See page 32.)

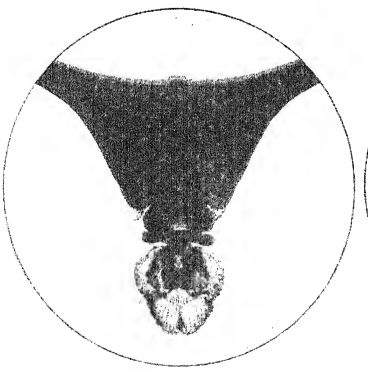


FIG. B.

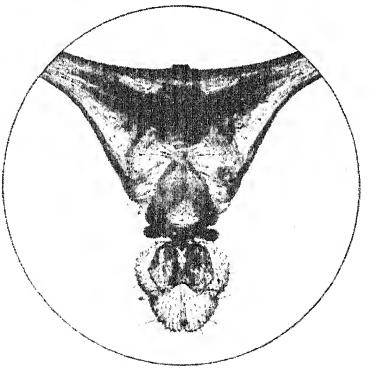


FIG. C.

Insect Preparation—Part of Head of Telescope fly—*Diopsis apicalis* x 22.

Fig. B. "C" Filter to obtain maximum contrast.
Fig. C. "F" Filter to obtain maximum detail.

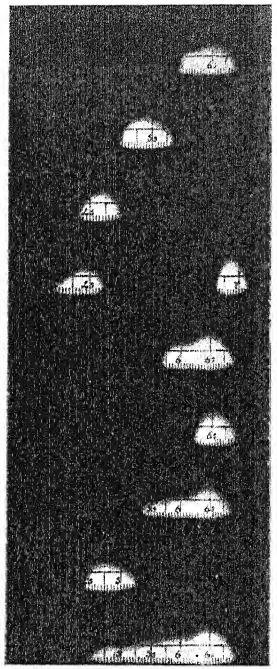


FIG. D.

Wedge Spectra of Wratten "M" Filters.

The above figure shows that this set of Filters with the Eastman Panchromatic Film affords complete control of the contrast when photographing preparations of any colour.

Wedge Spectra of Wratten "M" Filters, Combinations of "M" Filters, Visual "M" Filters and Combinations, and of Wratten Monochromat Filters and Mercury Monochromats.

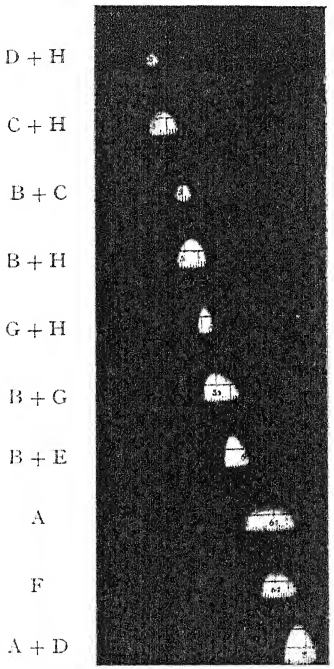


FIG. E.

Wedge Spectra of Combinations of Wratten "M" Filters.

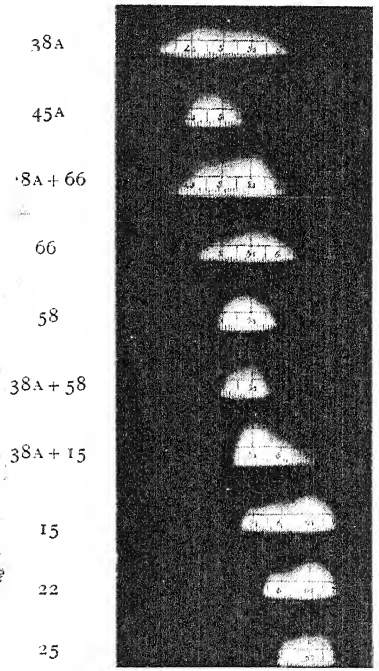


FIG. F.

Wedge Spectra of Wratten Visual "M" Filters and Combinations.

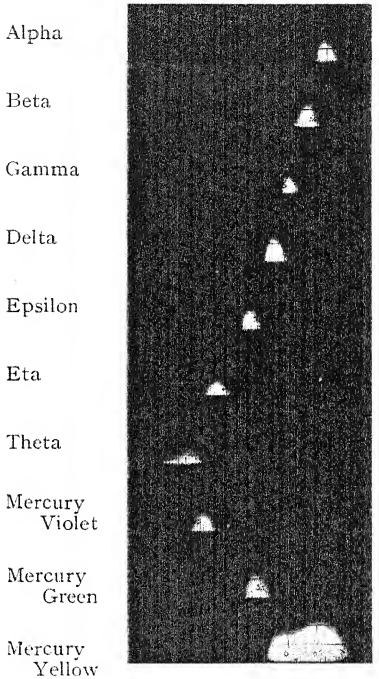


FIG. G.

Wedge Spectra of Wratten Monochromat Filters and Mercury Monochromats.

Set of 7 Wratten Monochromatic Filters—each Filter transmits a band of about 500 Angstroms in width.
Set of 3 Mercury Monochromats.

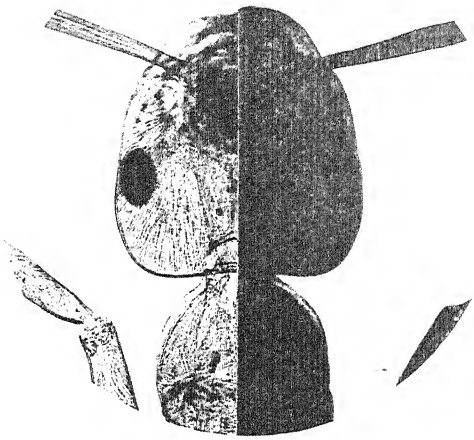


FIG. H.

Photomicrograph of Head of Wood Ant x 14, showing control of contrast and detail.

Right. With Blue "C" Filter.
Left. With Red "A" Filter.

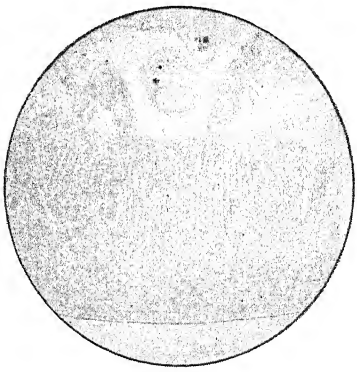


FIG. J.

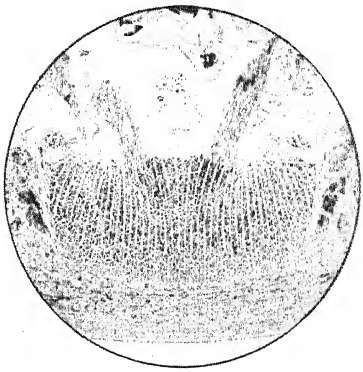


FIG. K.

Photomicrographs of a faint pink-stained T section of earthworm x 15.

Fig. J. on Process Plate.
Fig. K on Panchromatic with G.H. Filters.

case may be, is obtained. As a general guide, when contrast is required,

Use for	Blue-stained	Preparations	a Red filter
,,	Green ,,	,,	,, Red filter
,,	Red ,,	,,	,, Green filter
,,	Yellow ,,	,,	,, Blue filter
,,	Brown ,,	,,	,, Blue filter
,,	Purple ,,	,,	,, Green filter
,,	Violet ,,	,,	,, Yellow filter

When selecting a filter, care must always be taken that too much contrast is not obtained or the result will be a choking of shadows and loss of detail. It will be seen from the Table on the previous page that a very considerable choice of dominant wave lengths is available.

Sometimes better results are obtained by the use of much wider regions of the spectrum; thus, the B filter alone may be preferable to the B and G filters combined.

Dr. A. C. Coles, in his "Critical Microscopy" (1921), recommends the use of our No. 56 filter, in place of the B-G combination noted above. This can be supplied in all usual sizes from stock.

The K1 Filter

This filter should be used when incandescent gas or thorium pastille is the illuminant and it is required to reproduce coloured preparations, heat tinted sections, or objects under polarized light, in their approximately correct luminosity values. It will also serve when an oil lamp is the light source. If daylight or equivalent daylight be employed, the K2 or K3 filter should be used. The K1 filter is supplied in the "M" set of filters in the absence of other instructions and will be sent when a yellow filter for photomicrography is ordered.

For a discussion of the subject of correct rendering of luminosity values, "The Photography of Coloured Objects" should be consulted.

Microscopic Stains and Suitable Filters

The following list of some of the chief microscopic stains and their absorption bands, with the filters recommended to obtain maximum contrast, will be found useful.

It may be noted here that, except in the cases of the very dark combinations, these filters may be usefully employed in the visual examination of faintly stained preparations. But where light-filters are required solely for visual work we recommend our Visual "M" set. (See p. 27.)

Stain	Spectral Absorption Bands	Filters	Band used
Aniline Blue	5,500-6,200	B & E	5,600-6,000
*Bismarck Brown	General in Blue	C	4,000-5,100
Carbol Thionine	5,500-6,000	B & E	5,600-6,000
Congo Red	4,800-5,200	B & C	4,600-5,100
Cotton Blue	4,950-5,500	B & H	4,900-5,400
Eosine	4,900-5,300	G & H	5,100-5,400
Erythrosine	5,100-5,400	G & H	5,100-5,400
Fuchsine	4,800-5,700	B & G	5,100-6,000
Gentian Violet	5,700-6,000	B & E	5,600-6,000
Giemsa	General in Green	B & G	5,100-6,000
Gram	General in Green	G & H B & G	5,100-5,400 5,100-6,000
Hematoxylin :			
(Ehrlich)	Gradual through Green	B & G	5,100-6,000
(Delafield & Heidenhain)	5,600-6,000	B & E	5,600-6,000
Iodine Green	6,200-6,500	F	6,100-6,800
Leishman	General in Green	B & G	5,100-6,000
Methylene Blue	6,000-6,200 & 6,500-6,800	D & G	6,400-6,800
Methyl Violet	5,800-6,000	B & E	5,600-6,000
Methyl Green	6,200-6,500	F	6,100-6,800
Picro-Carmine	5,100-5,300 & 5,600-5,700	G & H	5,100-5,400
Rose Bengal	5,300-5,600	G & H	5,100-5,400

* When photographing by transmitted light, for insects and yellow sections generally, photograph for *contrast*, with a C filter; for *detail in the section*, with an F filter.

Unstained Preparations

In photographing unstained preparations of slight contrast, as, for instance, diatoms and fibres, use the Eastman Process films, and develop according to the instructions enclosed in each box.

Figs. 8 and 9, made from spectrophotometric measurements, show the method by which the preceding table of stains and filters is constructed.

A strong solution of Fuchsine gives an absorption band stretching from about 4,800 to 5,700. Fig. 8.

By the rule given on page 17, to obtain maximum contrast with sections stained with this dye we should use a filter, or combination of filters, having a transmission band similar in position to the absorption band of the dye, namely 4,800-5,700. We find, however, that except in the case of very faintly stained preparations, it is better to use filters whose transmission band is not centrally placed as regards the dye absorption band but is slightly to one side, otherwise an excess of contrast, or a blocking of detail will result. So, in the example given, instead of filters G and H transmitting between 5,000 and 5,500, we recommend the use of B and G 5,100-6,200. Fig 9.

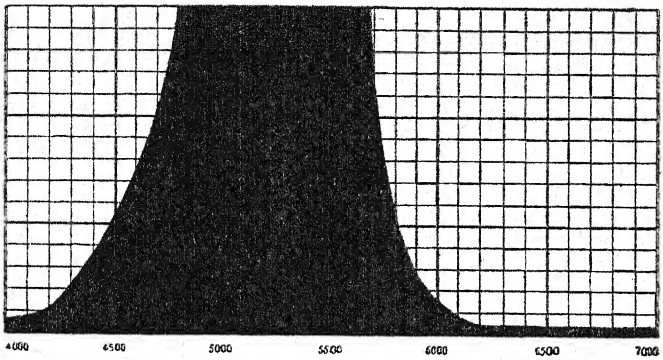


FIG. 8

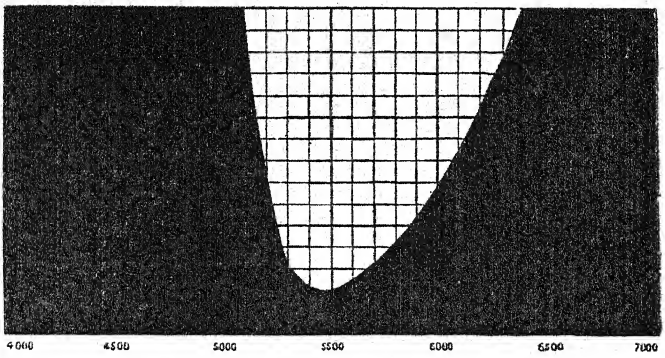


FIG. 9

THE RELATION OF THE COLOUR OF THE LIGHT TO THE RESOLVING POWER

The distance between two lines which can just be resolved by a microscope objective is equal to half the wave-length of the light by which it is observed, divided by the numerical aperture of the objective.

$$D = \frac{1}{2}\frac{w.l.}{\text{N.A.}}$$

We see, therefore, that the shorter the wave-length of the light the better the resolving power.

Thus, with an N. A. of 1·0 and an F Filter, we should be able to see 80,000 lines to an inch, with a B filter, about 95,000 lines to an inch, and with a C filter (if we get enough light) 100,000 lines to an inch.

Thus, with the C filter, negatives can be made showing dots in the diatom Amphipleura pellucida which are 100,000 to the inch, while these are invisible with a filter of longer wave-length.

The H filter will be found very useful in visual work for increasing the resolving power and if an arc is available even C may be employed. (See p. 29.)

PHOTOMICROGRAPHS OF METAL SECTIONS

We are frequently asked as to the best way to photograph metal sections, and since metallography has been defined as " the study of the constitution and structure of metals and alloys and their relation to physical properties," a knowledge of the photomicrography of opaque material is necessary. Essentially there is little difference between this class of work and ordinary photomicrography of transparent material. The main difference consists in using vertical illumination and objectives corrected for use without cover-glass and of short mount.

To obtain vertical illumination, a prism or plain glass illuminator is inserted between the objective and the nose of the microscope. The former gives the more brilliant illumination, but with objectives of higher power than four millimetres, the cover-glass illuminator gives a sharper image. Particular care should be taken to select a good flat cover-glass. In a number of cases of trouble from poor definition the cause has been traced to a wavy cover-glass.

Mounting the Specimen

Having set up the microscope, the beam of light is focussed into the opening of the illuminator which is then adjusted so that the specimen is evenly illuminated. As the beam of light remains fixed, the position of the illuminator must not be changed, and it is therefore necessary to use either an adjustable stage or a mounting device which compensates for the variation in thickness of different specimens. A convenient and simple mount consists of a brass cylinder, about 2 inches in diameter and 1 inch deep, which has been brazed to a thin brass plate about $3 \times 2\frac{1}{2}$ inches. In the bottom of the cylinder a lump of modelling clay is placed, the specimen stuck on top and carefully pressed down by a piece of plate glass until the latter rests on the rim of the cylinder. The surface of the specimen is then parallel with the base of the mount, and when placed on the stage is perpendicular to the axis of the microscope.

To focus the image sharply the transparent centre of the ground glass screen of the camera is used and the final focussing done by means of a magnifier.

For low-power work, say up to 100 diameters, using a 16 or 25 millimetre or a $\frac{2}{3}$ or 1 inch objective, incandescent gaslight gives sufficient illumination for a reasonably short exposure. For higher powers, a small arc light is better.

With a 2 millimetre apochromatic objective and a Wratten B filter, sharp photographs can be obtained on the Eastman Panchromatic film and the small arc light with, say, 10 seconds exposure for 1,000 diameters. Some workers prefer the use of the G or C filters.

For heat-tinted specimens, the K1 filter should be used with the same film.

THE WRATTEN VISUAL "M" FILTERS

are primarily intended, as the name implies, for visual work with the microscope. The set described below usually consists of nine circular filters, which we have found are the most generally useful.

Number	Colour	Use
78	Blue	Perhaps the most valuable filter made for visual use. Primarily intended for the conversion of the light from metal-filament vacuum lamps to equivalent daylight, it is also of great use with other light sources, such as the new thorium pastille gas lamp and especially with the customary oil lamp. It does not alter the real (daylight) colour values and contrasts of a preparation, and enables long continued observations to be made without causing eye-strain.
38A	Blue	A filter for increasing the apparent contrast in faintly stained yellow or orange preparations. Helps in the resolution of fine detail.
45A	Blue-Green	Especially useful when the highest resolving power visually possible is required, as in the study of diatom structure. It has no red transmission and its dominant wave length is at about 4,700 A.U.
66	Light Green	A contrast filter for use with pink and red-stained preparations. Preferred by some workers for general use in place of No. 78.
58	Green	A contrast filter for use with faintly stained pink or red preparations.
15	Yellow	For increasing the contrast in blue preparations and for helping in the observation of detail in insect mounts by reducing the contrast between the preparation and the background.
22	Orange	
25	Red	Contrast filter for use with preparations stained with Methylene Blue, Methyl Green, etc.
96	Neutral tint	A filter for moderating the intensity of the illumination. The density supplied transmits about one-tenth of the incident light. (Other densities may be had, see page 25.)

The Wratten Visual " M " Filters are stocked in two sizes $1\frac{3}{8}$ inch (35 mm.) diameter circular, and $1\frac{3}{16}$ inch (30 mm.), diameter circular. The thickness of these filters does not exceed 2·7 mm. (about $\frac{1}{9}$ inch). The larger size is designed for use in any standard substage ring sufficiently deep to take the specified thickness. The smaller size is for use in a light metal cell (supplied with the set), intended to fit loosely over the eyepiece (uncapped student's pattern) to meet instances where :

(1) The substage stop-carrier is not deep enough to carry even the thinnest cemented filter, or

(2) There is no substage fitting, or

(3) The substage ring will carry one filter, but it may sometimes be desired to use two cemented filters, either

  (*a*) to obtain extreme contrast in stained preparations by means of a very narrow transmission band, or

  (*b*) to employ a neutral tint filter to cut down excess of light when a contrast or other filter is already employed in the substage ring. (Note.—It is usually possible to employ two filters together in the " above eyepiece " fitting.)

(4) In the examination of metals or other opaque preparations, where methods of illumination, such as those introduced by Watson and Silverman are used.

The use of this fitting is obviously restricted to work when the microscope does not approximate to the horizontal position.

It is not recommended that this " above eyepiece " method should be adopted when the highest powers and most perfect optical means, such as 2 mm. apochromatic objectives, are employed. But with ordinary achromats and medium-power eyepieces with powers up to the order of ×500 this method of fitting will be found very useful.

The control of colour contrast obtainable with this set of filters is indicated in the following table, which gives the filters and, in three cases, pairs of filters, arranged in the order of their dominant wave-length.

Filter	Dominant W.L.	Filter	Dominant W.L.
	Å.U.		
45A	4,700	66	5,500
38A	5,000	38A with 15	5,550
38A with 66	5,250	15	6,000
58	5,350	22	6,300
38A with 58	5,450	25	6,500

All these filters, or combinations of filters, are sufficiently bright for comfortable use with the usual $\frac{1}{2}$-inch wick oil lamp and bull's-eye condenser.

When the highest possible resolution visually attainable is required, and an arc lamp is available, the Wratten Filters No. 48C (blue), or 50L (violet), recommended by Dr. Spitta, in the third edition of his "Microscopy," should be used. Either may be substituted for any of the filters in the regular Visual "M" set.

The Wratten Neutral Tint Filter No. 96 is now available in nine varieties, transmitting 50%, 25%, 12%, 10%, 5%, 3%, 1%, 0·1%, and 0·01% respectively of the incident light.

We occasionally have complaints to the effect that some microscopists are unable to use the darker of the filters in our Visual set. In such cases, it would be well to make sure that the user is not deficient in colour sensitiveness in that particular region. (About 4½% of the male population of this country is colour blind to a greater or less extent, usually to the red end of the spectrum).

It may be added that some workers habitually use even the 45a with an ordinary oil lamp and stand condenser. With any light source more powerful than this—say the thorium pastille—a light neutral filter is often needed by workers of normal colour perception.

Lighter grades of several of the regular visual filters can be supplied if required.

RHEINBERG COLOUR FILTERS

Very thin, cemented filters, rings and disks for the Rheinberg method of multi-colour illumination can now be supplied in twelve varieties, to fit the standard substage ring 1⅜ inch (35 mm.) *only*.

The back lens of an ordinary Abbe condenser is very suitable for this striking method of colour illumination. The colour used for the centre determines the colour of the microscope field and the colour used for the ring that of the object. The colour of the ring should be brighter than that of the centre. White with blue centre, and red with dark blue centre, are amongst the best combinations. The black disk on a coloured ring is also very effective.

VARIETIES AVAILABLE

(1)—Greenish Blue disk for single use
(2)—Blue disk for single use
(3)—Green disk for single use
(4)—Red disk for combined use
(5)—Purple disk for combined use
(6)—White Matt centre disk
(7)—Black centre disk
(8)—Red ring for combined use
(9)—Orange ring for combined use
(10)—Blue Green ring for combined use
(11)—Blue ring for combined use
(12)—Red and Blue Sector Stop

COLOUR PHOTOMICROGRAPHY

Photomicrographs in colour can, of course, be made by the usual processes of colour photography; thus, our A, B and C filters constitute our regular tri-colour filters, and if three negatives are taken through these three filters and then printed by any of the usual colour processes a result in natural colours will be obtained, but inasmuch as most sections contain not more than two colours, good results can be obtained by two colour photomicrography.

The method of work is to choose filters which absorb one of the colours as much as possible and transmit the other, the two filters being necessarily complementary to the colours used for the stains, and after making two negatives through these two filters, to make prints in bichromated gelatine as explained in the paragraph on page 37, and stain the prints from the negatives in colours corresponding to the two sections.

Thus, suppose a section is stained red and green. Make two negatives on panchromatic film, one with a red filter, which will cause the green to appear clear in the negative and will not record the red, and the other with a green filter which will cause the red to record and not the green. Then make prints from these in bichromated gelatine, staining the print from the red negative with the original green stain and then that from the green negative with the original red stain and superimpose the prints. It will be found that lantern slides made in this way will reproduce the original appearance of the slide in a very satisfactory manner.

The choice of the filters is, of course, of critical importance, and this is best decided by visual trial under the microscope, the filters chosen being those which most nearly absorb one colour and transmit the other. A few examples may be of value: Photographing a section stained with Delafield's Hematoxylin and Precipitated Eosine the A filter shows no trace of the Eosine and gives a good strong negative of the Hematoxylin. The B and C filters together record the Eosine and Hematoxylin both fully, and when the positives are made and stained with a blue dye and a red dye the resulting lantern slide will be excellent. For photographing Methylene Blue and Eosine the F filter and B and C together give good results. With Basic Fuchsine and Aniline Blue the A and C filters are satisfactory.

It should be mentioned that in making negatives for printing in bichromated gelatine a much softer negative must be made than for printing on paper or lantern plates; that is, the time of development should not be above two-thirds of that required for normal contrast. This is very important. The exposure should also be on the short side in order to secure transparent negatives. The exposure ratio too is of importance. When

this is correct neutral coloured portions of the section will show the same amount of detail in both negatives.

PHOTOGRAPHIC PROCEDURE

When the filters used transmit blue, green, or yellow light, very good results can be obtained on the Eastman Commercial Ortho Film or other orthochromatic material, but when orange or red filters are required it is necessary to use panchromatic material, and we recommend the Eastman Commercial Panchromatic Film for this purpose. These Panchromatic films being sensitive to red, no red light can be used in the dark-room and the subject of dark-room illumination will be found discussed on page 34. These films are flat, of stout substance, and are handled singly, like plates.

Exposure

To alter an old saying slightly, of the making of methods for estimating the exposure required in photomicrography there is no end! If one is confined to a very narrow range of subjects and magnifications little difficulty will be met as regards correct exposure. But if even a moderate range of subjects and magnifications has to be dealt with several variables come into the estimate. We have, e.g., the light source, more or less constant, and the method of using it, whether for axial, oblique, dark ground, or direct illumination; the aperture of the collecting lens and of the substage iris, the colour of the light and of the object, the working aperture of the objective, the degree of magnification, the speed of the plate, and factor of colour-filter if one is used; the class of object and the degree of contrast required.

One of the most direct methods, involving the measurement of the diameter of the Ramsden disk, was described by Dr. D. J. Reid before the Royal Photographic Society in December, 1908. Another method is to find out experimentally the exposure needed under known conditions for a typical object, say an average diatom group, a thin section, or well-cleared insect preparation, at a magnification of, say, 100 diameters, and call this length of exposure the standard exposure. Then by means of the tables given below—Effect of N. A. (note particularly that the *working aperture* of the objective is required), and Effect of magnification, it will be possible to calculate the approximate exposure needed for other conditions. The multiplying factor for colour-filters, if used, must also be taken into account. For instance, assume the standard exposure to be 10 seconds with an (actual) N. A. 0·5, 100 diameters, no filter, oil lamp, and we wish to photograph a section with a 1-inch objective (N. A. 0·25) at 50 diameters using a B light-filter factor 18, oil lamp, we get the following calculation:

$$\text{Standard Exposure} \times \text{Factor for N.A.} \times \text{Factor for Magnification} \times \text{Factor for Filter} = \frac{10 \times 4 \times 1 \times 18}{16} = 45 \text{ seconds.}$$

Another method, and one strongly recommended is to make a trial exposure after the following manner. A film of the usual size is taken and, for economical reasons, may be cut into narrow strips, a $\frac{1}{4}$-plate into, say, three or four. A series of progressive exposures is made, the length of the exposures to be varied in geometrical progression thus—1, 2, 4, 8, 16, 32, etc., seconds, for instance. A convenient way of effecting this is to cut a piece of stout cardboard into a series of steps (see Fig. A, page 19). To make this, the empty film holder is taken and the shutter drawn out to its full extent and the card cut to suit. The steps to be so arranged that when the card is placed against the shutter of the holder, after each increasing exposure, the steps will allow the shutter to be closed by a definite amount each time. So for eight exposures we should give first, with the shutter fully drawn, say, one second. Then close the shutter to the first step and give another exposure of one second ($1 + 1 = 2$), close to the second step and give two seconds ($1 + 1 + 2 = 4$) and so on, doubling the exposure each time, so that in this case the eighth exposure would be for 64 seconds, and the eight areas on the film would have received 1, 2, 4, 8, 16, 32, 64 and 128 seconds each respectively. From the strip of film then developed it should be easy to judge the best exposure.

If the film is much under-exposed try again, starting with, say one minute, and again doubling, but in this case a 4 step card should be sufficient with anything like efficient illumination, even with an oil lamp.

See that the shutter of your film or plate holder works easily.

Keep notes of all exposures, giving full details, particularly as regards working aperture of collecting lens, substage condenser and objective. After a representative series of exposures has been made and duly entered it will probably be found, either that the exposure in any fresh instance can be closely estimated with the help of the notes and the various tables given here, or, that a 4-exposure strip will be sufficient and save time. That is, if the exposure is thought to be, say, 15 seconds, give four exposures of 5, 5, 10 and 20 seconds, successively, equalling 5, 10, 20 and 40 on the film.

When a colour-filter is to be used place it in position before making the trial strip exposures. This will prevent any trouble arising from uncertainty of filter-factor or variation in the colour-sensitiveness of film employed, which might occur if the

nominal factor of the filter were applied to the unscreened exposure strip.

Effect of N.A.

Exposure varies as $\frac{1}{(\text{N.A.})^2}$

Equivalent focal length in.	mm.	Average N.A.	Approximate exposure factor
4	100	0·08	40
3	75	0·09	30
2	50	0·15	10
1	25	0·2	6
$\frac{3}{4}$	16	0·28	3
$\frac{1}{2}$	12	0·35	2
$\frac{1}{3}$	8	0·5	1
$\frac{1}{4}$	6	0·7	$\frac{1}{2}$
$\frac{1}{6}$	4	0·8	$\frac{2}{3}$
$\frac{1}{8}$	3	0·85	$\frac{1}{3}$
$\frac{1}{12}$ oil	2	1·0	$\frac{1}{4}$
$\frac{1}{12}$ oil	2	1·3	$\frac{1}{7}$
$\frac{1}{12}$ oil with oil condenser		1·0	$\frac{1}{8}$
$\frac{1}{12}$ oil with oil condenser		1·3	$\frac{1}{15}$

Note particularly that the factor to be used is the *working* aperture of the objective. This should be ascertained immediately before making the exposure, either by judging the proportion of the back lens which is filled with light and estimating the working aperture accordingly, or, more precisely, by measuring the diameter of the Ramsden disk in the manner suggested by Dr. D. J. Reid in a paper to which reference has already been made (p. 31).

The *numerical aperture* of a photographic lens employed for photomicrographic purposes, such as the short focus anastigmats much used for low-power work, is found by dividing the magnification by the product of (twice the f value and the magnification plus one). Thus, a lens working at $f6$ and magnification, say, four diameters

$$\frac{4}{6\times2\times(4+1)} = \frac{4}{60} = \frac{1}{15},$$

$\frac{1}{15}$ converted into a decimal fraction is approximately 0·067 = N.A. required. (This rule was given by Mr. A. E. Conrady in *Knowledge* 1903, p. 237.)

Note.—The f value of a microscope objective is found by dividing the total magnification by twice the N.A. For instance, say N.A. 0·7 magnification 350:

$$\frac{350}{0\cdot7\times2} = \frac{350}{1\cdot4} = f250 \qquad \textit{Conrady.}$$

Effect of Magnification.

Exposure varies as (Magnification)^2:

Magnification	Exposure Factor
10	$\frac{1}{100}$
25	$\frac{1}{16}$
50	$\frac{1}{4}$
100	1
250	6
500	25
1,000	100

FILTER FACTORS

Filter	Eastman Commercial Panchromatic Film				Eastman Commercial Ortho Film			
	Open Arc	Gas-filled and Pointolite.	Incandescent Gas, Thorium Pastille, Acetylene.	Oil	Open Arc	Gas-filled and Pointolite	Incandescent Gas, Thorium Pastille, Acetylene.	Oil
✓A	10	5	6	4				
✓B	16	16	12	18	24	24	20	15
✓C	7	20	20	30	6	8	9	10
✓D	Only	used in	Combi	nation	5	7	8	10
E	9	4	5	3	50	50	50	40
F	18	6	9	5				
G	8	3	4	2	40	30	30	8
✓H	7	15	20	20	6	6	7	8
K1	1½	1½	1½	1½	1½	1½	1½	1½
DH	60	100	120	180	50	100	120	150
CH	70	80	90	150	50	70	80	150
BC	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500
BH	250	250	300	300				
GH	1,200	1,200	1,200	1,200				
BG	40	30	30	30	30	25	25	30
BE	180	150	150	150	300	300	300	250
AD	80	50	80	40				
38A	5	5	5	6	3	2½	2½	2½
✓45A	8	18	20	24	7	8	9	10
66	3	3	3½	3	3	3	3	3
1. 56 55	6	6	5	6	8	6	8	9

DEVELOPMENT

Dark-Room Illumination

Since the Eastman Panchromatic film and any other similar panchromatic material is sensitive to red it is clear that the ordinary red dark-room lights cannot be used with such material. In their place we have prepared special green safelights (Series 3).

These are made on the following principle : the eye is sensitive to all colours if the light is sufficiently bright ; but as the intensity of light gets less, the eye becomes less sensitive to red and blue and proportionately more sensitive to green, until, if the light be very faint indeed, the middle of the green is by far the brightest light. Now, when developing a plate or film sensitive to all colours, we must use the light by which we can see the most with the least possible light, and this condition is realised with the Wratten safelights.

The safelights are made of a sheet of glass coated with a bright yellow gelatine film and another sheet coated with a bright green film put face to face with a thick sheet of green paper between. *No other safelights are safe for these red-sensitive films in the slightest degree, and red light especially must be avoided.*

When the white light is first turned out in a dark-room with a green safelight, even the safelight itself is scarcely seen, but in a few minutes most objects near the light will become plain, and at the end of fifteen minutes doubts will probably arise as to the safety of the now bright light.

When in 1907 the Wratten laboratory investigated the question of dark-room illumination in order to put on to a definite and scientific basis the ratio between the safety and intensity of dark-room lights, it was found to be very advantageous that dark-room lamps should work by reflected light only and that no direct light from the source should leave the lamp. In order to fulfil this condition and, at the same time, to obtain a lamp which could be relied upon the Wratten dark-room lamp was designed together with the Wratten Safelights. Safelight series 3 (green) should be used with all panchromatic material, and the series 2 (red) with Eastman Commercial Ortho film and all other similarly sensitive plates and films.

The Time of Development

The green safelight, although it is very much better than total darkness and is convenient to avoid mistakes in handling measures and dishes, must not be expected to give sufficient light to enable development to be watched except, possibly, after a long stay in the dark-room. Consequently development must be by time. We have, therefore, provided users of our Panchromatic Film with specific instructions as to the time for which they are to be developed. These instructions, which are enclosed in each box of the Film, are printed after actual tests in the laboratory have been made.

For timing development, either a clock with large hands or, more conveniently, a special time meter can be used. This clock can be set for a given time and then started in the dark, giving a signal at the expiration of the time.

Developing by Time

To develop a single film by time, proceed thus: get the dishes, developer, clock, etc., ready and have a cover ready for the developing dish (a larger dish will serve). Light the dark-room lamp with safelight. Put the developing solution into the dish. Observe the time or start the clock. Turn out the white light. Slide the Film into the developer, breaking any air-bells that may form on its surface. Cover the dish and rock gently for the time given on the card enclosed in each packet of Film. Pour the developer off and well rinse the film in clean water before transferring to the fixing bath which should, preferably, be of an acid and hardening character.

Caution.—The back as well as the face of the film is coated with gelatine so that neither side should be allowed to come in contact with anything during manipulation or while drying.

DEVELOPER

We recommend the following Pyro Soda formulæ:

STOCK SOLUTION "A."			
Water	16 oz.	or	500 cc.
Potass. Metabisulphite . ..	90 grains	,,	6 grams
Pyro	1 oz.	,,	30 grams
STOCK SOLUTION "B."			
Water	16 oz.	or	500 cc.
Soda Sulphite Anhydrous ..	1½ oz.	,,	45 grams
or			
Soda Sulphite Cryst.	3 oz.	,,	90 grams
STOCK SOLUTION "C."			
Water	16 oz.	or	500 cc.
Soda Carbonate Anhydrous ..	1¼ oz.	,,	37.5 grams
or			
Soda Carb. Cryst.	3¾ oz.	,,	112.5 grams

For use take 2 dr. A, 2 dr. B, 2 dr. C and add 14 drams of water.

For normal contrast develop for the times given on the card in each box of Panchromatic film.

If a two-solution formula is preferred, the following may be used. Develop for one half the time given on the card:

(1)	Water	60 oz.	or	1,000 cc.
	Soda Sulphite Cryst.	6 oz.	,,	100 grams
	Pyro	1 oz.	,,	17 grams
	Pot. Metabisulphite	¾ oz.	,,	12 grams
(2)	Water	60 oz.	or	1,000 cc.
	Soda Carb. Cryst.	6 oz.	,,	100 grams

For use take equal parts of 1 and 2.

FIXING

An acid fixing and hardening bath is strongly recommended. The following formula may be used:

(*a*)	Water	48 oz.
	Hypo	16 oz.
	Soda Sulphite Anhydrous	1 oz.
(*b*)	Water	16 oz.
	Chrome Alum	1 oz.
	Sulphuric Acid	1 dram

Pour B solution slowly into A solution while stirring A rapidly.

If a plain acid fixing bath is preferred, use:

Water	40 oz.
Hypo.	10 oz.
Potass. Metabisulphite	1 oz.

Always use fresh, clean and strong fixing baths, as the gelatine coated back of the films is likely to become stained in old and discoloured solutions.

For paper prints we recommend Kodak Nikko paper, Vigorous (glossy surface) for the average subject, or Velox Glossy if greater contrast is required. If a velvet or a matte paper is required, use Soft Art Velox or Kodak Platino-Matte Bromide.

The utmost detail can also be rendered on a printing-out paper such as "Solio," particularly if this is dried after the final washing on a ferrotype plate or piece of plate glass previously talced.

For transparencies or lantern slides any of the usual makes of Lantern plates are satisfactory, but undoubtedly the best method of making lantern slides from the great majority of microscopic subjects is by printing in bichromated gelatine, and staining the gelatine relief with dyes as described in the next section, since this method gives slides which approximate very closely to the appearance of the original section.

Making of Slides in Bichromated Gelatine

Lantern plates are sensitised by bathing for five minutes in a 2½% solution of ammonium bichromate containing 5 cc. of ·880 ammonia to a litre. The sensitising may be done by artificial light as the plates are practically insensitive when wet. The temperature of the bath must not be above 65° Fahr. or reticulation of the gelatine is very likely to occur. The bath may be used repeatedly but should be filtered before use.

The plates as they come from the sensitising bath are rinsed for two or three seconds in clean water to remove superfluous bichromate solution, and are then carefully drained in a rack

until the whole surface is free from streaks, after which the backs are cleaned and drying hastened by a gentle current of dust-free air from a fan. The current of air must not be violent otherwise streaks of uneven sensitiveness will occur. A gentle current of air, which will dry the plates in about an hour is very satisfactory. Drying must take place in darkness or in a safe orange light.

The plates will remain good for several days if stored face to face in plate boxes.

Printing the Plates

The plates are exposed *through the glass* under a negative to the light of an arc lamp, preferably with $\frac{1}{4}$ inch iron rod as electrodes in place of the usual carbons. With the type of negative best suited to this class of work the average exposure with such an iron arc, taking about 5 amperes, and printing at 10 inches distance, would be about two minutes. With ordinary carbon poles about $1\frac{1}{2}$ times as long. The printing cannot be done by daylight, as when printing through the glass there will be too much diffusion to give sharp images.

The plates are developed by rocking in trays of water at about 120° Fahr. until *all* soluble gelatine is removed. Under exposure is indicated by the high-light detail washing away, and over-exposure by the film being insoluble to too great a depth.

The plates are next rinsed in cold water, fixed in acid hypo and washed free from the hypo ; they are then ready for staining.

Throughout the foregoing operations the delicate film must not be touched with anything other than liquids.

Staining

For staining, a 1% solution of dye is used, adding acetic acid equal to 1% of the volume of the dye bath, the dye being selected to simulate most closely the original stain of the section. The time of dyeing is not important, but must be long enough to saturate the gelatine reliefs. The following are suggestions for the imitation of some of the best known stains, though in slides made as suggested on page 30, from two negatives the dyes must be selected so that in combination they most closely simulate the original section :

Stain	Suggested Dye
Aniline Blue	Pinatype Blue F (Hoechst)
Congo Red	Naphthol Red
Eosine	Eosine or Naphthol Red
Delafield's Hematoxylin	Water Soluble Nigrosine
Basic Fuchsine	Fast Red B.
Leishman	Water Soluble Nigrosine
Methylene Blue	Toluidin Blue
Picric Acid	Chrysoidine S

These are to be regarded only as suggestions, and a little experimental work will soon show which dyes will give the best results. If necessary, the dyes can be washed out in dilute ammonia and another dye tried.

A method of obtaining a green colour simulating methyl green is to tone an ordinary black lantern slide in a vanadium toning bath. The following formula has been found satisfactory :

Ferric chloride	10 grains or	1 gram.
Oxalic acid (sat sol)	10 drams	50 cc.
Vanadium chloride	20 grains	2 grams.
Nitric acid	50 minims	5 cc.
Water up to	5 oz.	200 cc.

After dissolving, add the following solution, a little at a time shaking constantly :

Potass. ferricyanide	50 grains	5 grams.
Water	5 oz.	200 cc.

Immerse plate in bath from one to two minutes according to tone desired. The longer a plate is left in the bath the lighter the tone will be.

When printing two-colour slides from double negatives, one of the negatives must be reversed so that the film is towards the light ; thus, we shall have in printing, first the negative film, then the back of the negative film in contact with the glass of the lantern plate and, finally, the bichromatised positive emulsion. In order to get sharp results the iron-arc, previously described, is strongly recommended.

This is a case where the use of Film in negative making is a great advantage over glass plates, as with the latter the necessary reversal causes a considerable amount of unavoidable diffusion and trouble in registration. The use of a condenser in a projection lantern with the projection lenses removed is the best way of minimising this trouble, but there will then be little advantage in using iron poles over the usual carbons, as the excess of ultra-violet from the iron arc will be largely absorbed by the thick condenser lenses.

The reversal of the one negative is necessary in order to be able to mount the dyed plates film to film to make the slide.

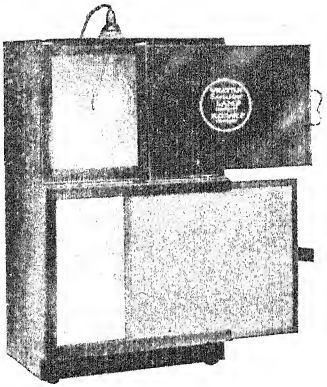
PRICE LIST.

	£ s. d.
WRATTEN "M" FILTERS	
Set of 9—2 in. square, cemented	2 18 6
,, 12—2 in. ,, ,, ...	3 18 0
,, 9—4 in. ,, ,, ...	8 8 9
,, 12—4 in. ,, ,, ...	11 5 0
Single Filters, 2 in. square	0 6 6
,, ,, 4 in. ,, ...	0 18 9
VISUAL "M" SET FOR SUBSTAGE RING	
1⅝ in. Circular Cemented Set of 9	2 5 0
VISUAL "M" SET EYEPIECE TYPE	
1$\frac{3}{16}$ in. Circular Cemented Set of 9 with Cell	2 5 0
Single Filters, 1⅜ in. circular	0 5 6
,, ,, 1$\frac{3}{16}$ in. ,, ...	0 4 6
Cell only	0 3 6
SET OF NINE GELATINE FILMS	
1 in. square	0 2 6
1½ in. ,, ...	0 5 9
2 in. ,, ...	0 10 0
SPECIAL "M" STAND	
To hold one or two 2 in. square Filters	0 17 6
,, ,, ,, 4 in. ,, ,, ...	2 0 6
RHEINBERG COLOUR FILTERS	
Rings and Disks, cemented 1⅜ in. circular, 12 varieties ..	3/- each
Set of 12	£1 13 6

FILMS	Panchromatic.	Commercial Ortho & Process.
Size :	s. d.	s. d.
3½ x 2½	2 6	1 8
4¼ x 3¼	3 9	2 6
5 x 4	5 0	4 2
6½ x 4¾	7 6	5 6

	£ s. d.
KODAK PYRO SODA DEVELOPING POWDERS	
Per box of 6	0 1 6
KODAK ACID FIXING SALT	
Per ½ lb. tin	0 0 10
,, 1 lb. ,, ...	0 1 6
WRATTEN SAFELIGHT LAMPS	
Junior, for Nightlight, complete with one Safelight ..	0 4 6
7 x 5, for Gas, Oil or Electric Light, complete with one Safelight	1 0 0
10 x 8, for Gas, Oil or Electric Light, complete with one Safelight	1 10 0
12 x 10, for Electric Light only	2 0 0
EXTRA SAFELIGHTS	
7 x 5 .. .. each	0 4 0
10 x 8 .. .. ,,	0 5 6
12 x 10 .. .. ,,	0 8 6

K.—40274



The Wratten Safelight Lamp

The Wratten Safelight Lamp gives the maximum amount of illumination with the highest degree of safety. No direct rays whatsoever come from the Lamp—the light is all reflected light.

The lamps can be supplied for use with Electricity, Gas or Oil, and are used in conjunction with the Wratten Safelight. These Safelights range from a clear yellow for use with Gaslight Paper, to a dim green or use with Panchromatic Film.

The Lamps are made in three sizes :

7 × 5 in.	complete with one Safelight					-	£1	0 0
10 × 8 in.	,,	,,	,,	,,		-	£1	10 0
12 × 10 in.	,,	,,	,,	,,		-	£2	0 0

Kodak Limited, Kingsway, London, W.C.2.